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Professor Rainer Glaser, Associate Editor
321 Chemistry Building
601 S. College Avenue
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RE: REVISED
Hexadecyltrimethylammonium Bromide – A Novel Cationic Detergent
By Lance A. Schell and Jedidiah D White

Dear Dr. Glaser:

Thank you for your communication of April 18 with the peer reviews of our original manuscript, *Hexadecyltrimethylammonium Bromide – A Novel Cationic Detergent*. We have made changes based on peer reviewer comments as well as on our own accord. The comments from the peer reviewers and the changes we have made are as follows:

Major Revisions

[M.1] Reviewer 6 writes, "… the introduction [should] be reworked with more focus if possible." The introduction was substantially rewritten with an increased focus on the nanoparticle application of our surfactant. An additional application (SDS-PAGE) was also added. To incorporate this change, substantial edits were also made to the abstract and conclusion. Specifically, acute respiratory distress syndrome and many elements of the introduction mentioned in the review have been incorporated throughout the paper better.

[M.2] Reviewer 6 writes, "… the paper would benefit from an additional paragraph … that describes the manufacturing process of the gold nanoparticles." An additional section detailing this process has been added to the "Materials and Methods" section.

[M.3] Reviewer 6 writes, "It would support your conclusion if the pictures in Figure 4 (former) could be compared to images of gold nanoparticles produced by a manufacturing process with some other surfactant." These images have been incorporated as part of the introduction rewriting (see **[M.1]**).

[M.4] A graphical abstract was added to the end of the abstract as required in §5 of A11.

[M.5] Additional analysis was provided for SDS-PAGE techniques. The gold nanoparticle results section was rewritten with much more detail and focus to mirror the new introduction. Section headers have also been added to the "Results" section for better text navigation.

[M.6] During review of the *Journal of Organic Chemistry Guidelines for Authors*, the manuscript was changed in terms of page margins and adding blue lines (RGB=0:84:166) to accodomate *JOC* recommendations. A recent survey of a *JOC* article (Saravanan et al.) confirmed this style.

Response to Reviewer 6

Note About Spectra Suggestion: While the spectra could easily be removed and placed into the Supplementary Materials section, §2.2.2 of the *JOC* author guidelines states that the journal "… upholds a high standard for compound characterization to ensure … compounds … have been correctly identified." The journal wants establishment of both identity and degree of purity. The analysis of the specific peaks is given to satisfy the requirement of citing our literature used as a reference for interpretation of the NMR spectra and provide identity, while the spectra images themselves provide illustrations as to the degree of purity within our "new" compound. Hence, we have left the spectra images as they are in the "Materials and Methods" section.

[6.1] Figures and scheme captions were changed to Times New Roman and 12 pt. font as requested. The table caption was already Times New Roman, but was increased to 12 pt. font.

[6.2] Manuscripts submitted using *Paragon Plus* (the submission system for ACS journals, including the *Journal of Organic Chemistry*) are to be double spaced throughout. Through previous personal manuscript preparation experience and research on the ACS website, "extra" spaces are removed from the default *Microsoft Word* settings. Thus the options initially selected were "Remove Space Before Paragraph" and "Remove Space After Paragraph". Since the manuscript complies with submission guidelines in this regard, no change was made. Some authors do prefer to leave a space between sections, but we think it just takes up extra space.

[6.3] As per §2.1.2 of *Journal of Organic Chemistry Guidelines for Authors*, the symbols † and ‡ are used as superscripts to show institutional affiliations of the authors and were added to show institutional affiliation. An asterisk denotes a corresponding author and was added to both authors as both are corresponding authors.

[6.4] As per A11 §5, "Abstract and graphical abstract should appear on the first page of the revised paper right below the title, author line and affiliation information, and introduction starts on p. 2 of the paper." Therefore, no change was made in this regard.

[6.5] Figure 1 was moved closer to its location of first mention within the text body. This change was incorporated in the refocusing of the introduction (see **[M.1]**).

[6.6] This change is usurped by the rewriting of the introduction (see **[M.1]**).

[6.7] This change is usurped by the rewriting of the introduction (see **[M.1]**).

[6.8] This sentence was removed during the rewriting of the introduction (see **[M.1]**).

[6.9] Scheme 1 was redrawn such that part C was rotated horizontally, decreasing the height of the image as suggested.

[6.10] The reference to Scheme 1 under the Synthesis, Reagents, and Materials section was changed to accurately reference the synthesis shown in Scheme 2.

[6.11] The paragraph directly below Scheme 2 was rewritten with better wording. Please refer to that paragraph to see the change.

[6.12] The sentence was changed to "… agent will behave. It is an expression …"

[6.13] The sentence was changed to "… appropriate gas constant depending on the required units ~~used~~."

[6.14] "α_m values are found…" was changed to "Values of α_m are found…"

[6.15] "chloroform-d" is an abbreviation for deuterated chloroform, a common solvent for NMR spectroscopy. Readers may also be familiar with deuterated water (D_2O) or benzene (C_6D_6). Thus, no change was made neither in the text nor in the supporting information in this regard.

[6.16] The sentence was changed to "… expected that the area under the curve at 1.3 ppm should … ratio to the area under the curve for N-H bonds absorption…"

[6.17] The table was refitted to be within the new page margins (see **[M.6]**). Some aligning work and rearrangement of text was done to accommodate them increased size of the 12 pt. font.

[6.18] The sentence was changed to "Both ^{1}H NMR and ^{13}C NMR characterization (shown in results) as well as…"

[6.19] The *Journal of Organic Chemistry Guidelines for Authors* details citations denoted as (#) in the bibliography, and the numbering was left as is. As per §2.1.12 of the *Guidelines for* Authors, footnotes are used exclusively for explanatory purposes. Volume numbers were italicized and article titles were added. This citation format was cleared by Dr. Glaser. In the absence of automated citation software, footnotes provide the next-best alternative to numbering citations easily and are why we are using them in this course. The footnotes may then be formatted to the style seen in this work. A quick survey of a recent article (Saravanan et al.) also confirmed this style.

[6.20] "While somewhat on the longer side…" was changed to "While somewhat lengthy…"

[6.21] The "Additional References" label was shortened to "References". Citations provided were already what we are pretending to be our own.

[6.22] While the *Journal of Organic Chemistry* guidelines call for no titles, Dr. Glaser has required the use of titles in citations, which the authors were not aware of. Titles have been added to the citations as suggested. Volume numbers were also italicized. (see **[6.19]**).

Response to Reviewer 7

[7.1] The paragraph describing the basics of surfactants was removed entirely and incorporated within the rewriting of the introduction (see **[M.1]**).

[7.2] In the rewriting of the introduction, Figure 1 was moved up relatively close to the location where it is first mentioned in the body of the text (see **[6.5]**).

[7.3] Reviewer 7 writes "… you stated that it is assumed that nanoparticles are evenly distributed … either your particular particles are different, or you are proving the previous assumption false." and to state which is true. We believe the discussion regarding the nanoparticles in the new introduction usurps the need for this change (see **[M.1]**).

[7.4] Some topics were mentioned in the introduction and method section simply to give the paper a sense of structure. The idea was to present the hypothesis and arguments we use in the third paragraph of the introduction, and then show through the rest of the paper (namely the methods section) just how we arrive at those arguments and substantiate our claims. The third paragraph has also seen modification as a result of the rewriting of the introduction (see **[M.1]**), but the premise remains the same. The modification does somewhat incorporate this change.

[7.5] As the new introduction and a rewording of the synthesis description will state, other synthesis procedures are indeed possible. Separation of catalyst *is* generally difficult and this synthesis manages to avoid it. We detail some of the various methods to remove catalyst in these types of syntheses, many of which are very complex. Had we used a shorter reaction sequence initially, we believe that time would be lost during the complex catalyst-product separation method. While that will probably be longer than our initial synthesis time of 30 hours, even if they were the same, our synthesis method would still be advantageous in regards to the guaranteed higher yield because of not dealing with anything in the product step.

[7.6] The primary focus of the paper revolves around comparing HTAB with other surfactants. Since HLB, CMC, and aggregation number are performance characteristics that do exactly this, we believe a discussion of how these values were reached is essential to the methods section.

[7.7] We believe it can be reasonably inferred that the results of the equations are in the "Results" section. No explicit change was made, but wording was changed throughout between the equations to kind of "lead" the occasional reader who is not able to infer this…

[7.8] No change was made as per A11 §5 (see **[6.4]**).

[7.9] Fonts throughout the paper were changed to Times New Roman. In many cases, the font was already Times New Roman, but was not 12 pt. This was corrected (see **[6.1]**).

[7.10] The table was adjusted to fit within the new page margins (see **[6.17]**).

[7.11] The wording of the final paragraph was changed to infer "support" of the hypothesis rather than "proving it correct".

[7.12] As per §2.1.12 of the *Journal of Organic Chemistry Guidelines for Authors*, there is no mention of indentation as you mention. You are referring to a "hanging indent" which is used by some journals to directly align the second line of a given reference directly below the first. A quick survey of literature in *JOC* showed this not to be the case. References simply start with a space following (#) and have no hanging indent. Hence, no change was made in this regard. Volume numbers were italicized and titles were added as per Dr. Glaser's requirements, although they do not appear in *JOC* – that is the only difference (see **[6.19]** and **[6.22]**).

[7.13] The page numbers for the table of contents of the appendix were changed to include S before the page number as suggested.

Response to Reviewer 9

[9.1] The second author does not have a middle name and the letter is not an initial. Hence "Jedidiah D White" is warranted over "Jedidiah D. White".

[9.2] This sentence was removed as part of the rewriting of the introduction (see **[M.1]**).

[9.3] The description of the basics of surfactants was removed as part of the refocusing of the introduction. This change is no longer applicable since this section has been removed.

[9.4] Font was changed to Times New Roman (see **[6.1]**).

[9.5] Continuous paragraphs that started before a figure, scheme, or table, etc. and continue following the respective item are not generally indented. A quick survey of the *Journal of Organic Chemistry Guidelines for Authors* and a recent article (Saravanan et al.) revealed this also to be the case. Hence, no change was made.

[9.6] The sentence "*In vitro*… culture." shows a basic compound sentence with two distinct subjects and not a clausal conjugate of two ideas. Hence, we do not believe a comma is justified since compound sentences do not use commas.

[9.7] Instead of simply adding "its" after the word "and" we have reworded the sentence to "… results in an increased uptake by cells, which creates issues in the development … where minimizing negative response to drugs and using precise amounts for treatment are essential."

[9.8] The word "its" was incorporated into a change to sentence wording (see **[6.6]**).

[9.9] This sentence was removed as part of the rewriting of the introduction (see **[M.1]**).

[9.10] This sentence was removed as part of the rewriting of the introduction (see **[M.1]**).

[9.11] Font was changed to Times New Roman (see **[6.1]**).

[9.12] Font was changed to Times New Roman (see **[6.1]**).

[9.13] Font was changed to Times New Roman (see **[6.1]**).

[9.14] As per §2.4 of the *Journal of Organic Chemistry Guidelines for Authors*, "Above each table should be typed, in boldface characters, a sequential Arabic table number and a short descriptive title." While no change was made to the location of the title, the rest of the title was bold-faced to comply with these recommendations.

[9.15] The paragraph is continuous with the text before the table. Therefore no indent is necessary as the text is not intended to be a new topic (see **[9.5]**).

[9.16] We decided to remove the respective sentence in question and have provided a more detailed description around nanoparticles that we believe adequately satisfies the suggestion to talk about "other coatings".

[9.17] The abbreviation "HTAB" was defined for hexadecyltrimethylammonium bromide in the introduction on first use and was substituted for the full name of the detergent for each use thereafter.

[9.18] Spacing in the paper between words is a result of the justification function of *Microsoft Word*. As justification of text in the manuscript is mentioned in the *Journal of Organic Chemistry Guidelines for Authors* and this functionality is done automatically, there is no change that can be made regarding spacing. It depends on the number of words and letters per word used within each line of the text and spacing between any two specific words is inherently random.

Once again, thank you for your consideration of our paper. The peer reviewers offered abundant suggestions and showed high attention to detail, which was much appreciated during the revision process. We hope that the revisions we have made will improve our paper to the level of an accepted manuscript for publication in the *Journal of Organic Chemistry*.

Sincerely,

Lance A. Schell Jedidiah D White

Hexadecyltrimethylammonium Bromide: A Novel Cationic Detergent

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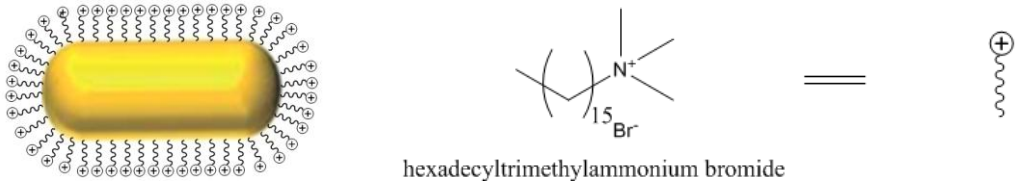
Abstract

The new cationic surfactant, hexadecyltrimethylammonium bromide, was produced using a synthesis procedure without the use of catalyst, allowing higher yield of product. Its ability as a potential surfactant was compared against sodium dodecyl sulfate and sodium deoxycholate monohydrate. The performance parameters hydrophilic-lipophilic balance, critical micelle concentration, and aggregation number were calculated for each of the surfactants and comparison was also made in applications in PAGE-based glycoprotein analysis and in the manufacturing of gold nanoparticles. Hexadecyltrimethylammonium bromide showed a much higher aggregation number as well as a stabilizing ability due to the attraction of opposite charges when complexed with glycoproteins. These two features allow this new surfactant to have a higher "association ability" with larger proteins. Finally, the wide applicability of hexadecyltrimethylammonium bromide was further demonstrated when gold nanoparticles coated with the surfactant showed similar features to dimethylformamide without carcinogenicity. This work seeks to establish hexadecyltrimethylammonium bromide with high potential as a new, novel, cationic detergent.



hexadecyltrimethylammonium bromide

Introduction

Detergents and surfactants have seen widespread use in many industries ranging from household laundry detergents and hygiene products to DNA extraction in biological sciences. Natural surfactant systems are also exhibited in the human body. A study by Poynter and LeVine analyzed the role of a surfactant system in the pathophysiology of acute respiratory distress syndrome; surfactant replacement therapy in patients diagnosed with such conditions led to improved oxygenation, lung compliance, and a decrease in the need for ventilator support.[1] Research in biological sciences heavily employs the use of detergents and surfactants. Sodium dodecyl sulfate (SDS) is used predominantly in SDS-PAGE analysis. SDS, along with the similar sodium deoxycholate monohydrate (SDM), is also used in studies concerning membrane-associated proteins. These surfactants break hydrogen bonding within the fatty lipid constituents of the cell membrane and lyse the cell, fostering the ability to extract DNA and RNA reliably from within the cell for analysis. SDS has seen extensive research use recently in immunoblot assays[2], while SDM has appeared in a study on HMG-CoA reductase inhibitors (i.e. pravastatin).[3] Finally, detergents and surfactants have found heavy use in the manufacturing of nanoparticles, which are becoming an increasingly popular medium for drug delivery and cancer treatment. Nanoparticles have also seen use in diagnostic lung imaging, such as that done for acute respiratory distress syndrome mentioned earlier.[4] These particles are unique in that their minute size does not generally induce an inflammatory response in the human body[4], yet their susceptibility to magnetic and electrical influence allows even the slightest modification(s) to impact their behavior. This is especially important in complicated imaging environments, such as the lungs or brain, where the behavior of the imaging agent must be tightly controlled. SDS-chitosan nanoparticles were recently studied as a potential carrier system for oral insulin[5], while

SDM-chitosan nanoparticles have seen use in studies involving plasmid delivery systems.[6] Gold nanoparticles have become highly desirable as they inherit special properties of gold atoms, which include high malleability, high ductility, sufficient conductivity, and inertness towards air and many reagents. The benefit most would be familiar with is the ability to make gold in various oxidation states using derivatives of auric acid. Figure 1A depicts gold nanoparticles in a particular rod-shaped morphology, which is the most frequently used form.[7] A TEM image showing gold nanoparticles (also in the nanorod morphology) is shown in Figure 1B.[8] Figure 1C depicts the clumping tendency of nanoparticles which will be discussed later.[9]

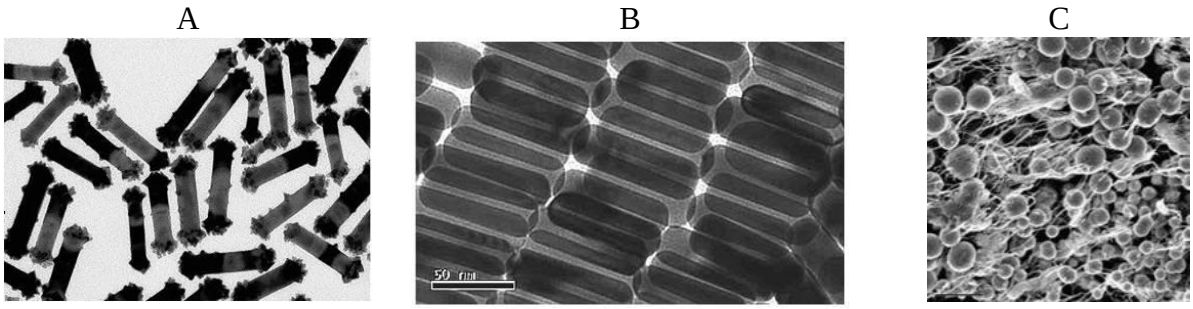


Figure 1. a) Gold nanoparticles in the nanorod morphology following production; b) TEM image of gold nanoparticles; c) Clumping tendency of nanoparticles seen in zinc-based nanoparticles

While surfactants are useful in a wide variety of industries, there are also many problems that must be addressed. First, synthesis of new surfactants generally poses a challenge as the catalyst often cannot be reused and is difficult to remove from the products. This problem has led to the development of complex methods involving catalyst removal with thermal structure modification[10] and the removal of the surfactant itself from the catalyst using carbon catalytic ozonation.[11] These methods require complex control of the reaction mechanism throughout the experiment. In the case of catalytic ozonation, the efficiency is somewhat stagnant in that, even with high concentrations of ozone and reagents, the decomposition efficiency remains unchanged.[11] Second, biological research frequently relies on the analysis of negatively-charged

glycoproteins. For instance, a study by Yang et al. provided a detailed analysis on over 150 glycoproteins found in human plasma.[12] When analyzed using existing SDS-PAGE techniques, bands are generally blurred due to repulsive forces between the parent SDS gel and the glycoproteins themselves.[13] Evidence of these blurred images and a discussion of a potential solution to this problem are discussed later in this work. Third, the use of SDS and SDM for lysing cell walls creates practical use problems. SDS is a relatively cheap surfactant, but requires a significantly larger amount when used in the presence of larger proteins due to its relatively low aggregation number. SDM is vastly more expensive than SDS (on the order of $1000 for 50 grams) and, while a better lysing agent, has an even lower aggregation number than SDS, limiting its use predominantly to smaller protein complexes relative to SDS. Aggregation number analysis is done later in this work. Finally, *in vitro* studies of nanoparticles expose cells to the nanoparticles at the bottom of a culture plate (as shown in Figure 2) and the assumption is made that the nanoparticles are evenly and thoroughly-distributed throughout the culture.[14]

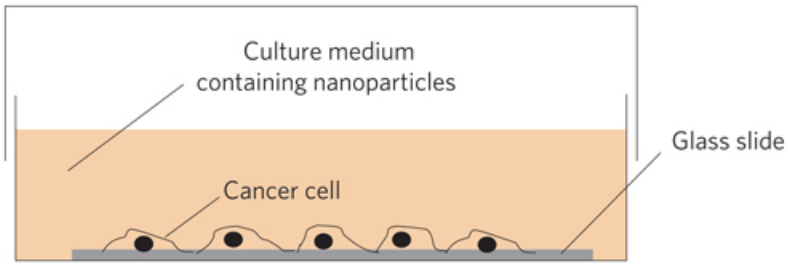


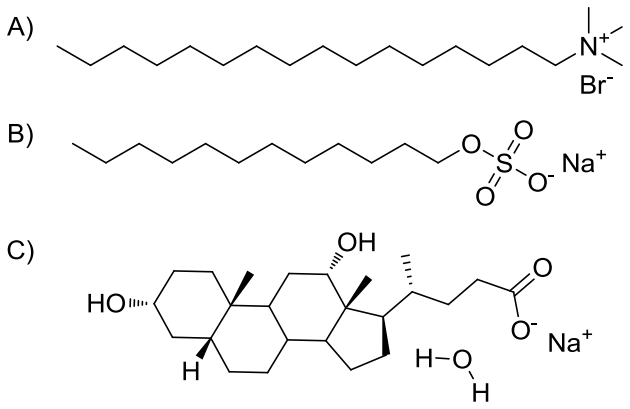
Figure 2. Diagram of an Experimental Setup for *In Vitro* Analysis of Nanoparticle-Based Drug Delivery

This assumption is likely not true due to the highly aggregative nature of nanoparticles. When additional nanoparticles are added to a suspension containing existing nanoparticles, they possess a tendency to clump together.[14] This aggregative behavior was shown in Figure 1C with zinc-based nanoparticles. Thus, the concentration of nanoparticles may be higher on the surface than

is actually present in the entire culture.[14] This results in an increased uptake by cells, which creates issues in the development of drug delivery systems where minimizing negative response to drugs and using precise amounts for treatment are essential. To prevent this aggregation, many manufacturers coat the particles in a particular surfactant depending on the constituent of the particle itself. A brief introduction to coatings was given earlier with chitosan complexes containing both SDS and SDM. Gold nanoparticles, in their current production development state, are coated with dimethylformamide (which is used in the images in Figure 1). This organic compound is a polar, aprotic solvent that is very unstable in both strong acid and alkaline environments. Application as a drug delivery system is thus limited, as the stomach (highly acidic) and intestine (highly alkaline) are primary targets for drug delivery. Dimethylformamide has also been listed as a carcinogen and may cause birth defects.[15] Nanoparticle development is further complicated by the behavior of nanoparticles following binding to the target surface. Upon binding, the nanoparticles become negatively-charged and their behavior is extremely sensitive to their orientation. Use of anionic surfactant coatings with the negatively-charged nanoparticles upon binding would possibly destabilize the particles, resulting in extreme sensitivity to orientation, and loss of control of their action(s), which is not desirable in many drug delivery scenarios.

Here we report a synthesis procedure for a new surfactant, hexadecyltrimethylammonium bromide (HTAB), which is unique within surfactant chemistry in that it does not involve a catalyst. While HTAB is the systematic IUPAC name, the name cetrimonium bromide is synonymous with our surfactant when using common nomenclature. We will investigate HTAB as a potential cationic surfactant using the calculated performance parameters hydrophilic-lipophilic balance (HLB), critical micelle concentration (CMC), and aggregation number. We

will further investigate the applications of HTAB in SDS-PAGE analysis in biological science research and in the manufacturing of gold nanoparticles. Since SDS and SDM are used in highly similar fashion in the areas we intend to evaluate, they will be used for comparative purposes throughout this work. Structures for HTAB, SDS, and SDM are shown in Scheme 1.



Scheme 1. Structures of a) Hexadecyltrimethylammonium Bromide (HTAB) and Other Commonly Used Detergents in Biological Sciences – b) Sodium Dodecyl Sulfate (SDS) and c) Sodium Deoxycholate Monohydrate (SDM).

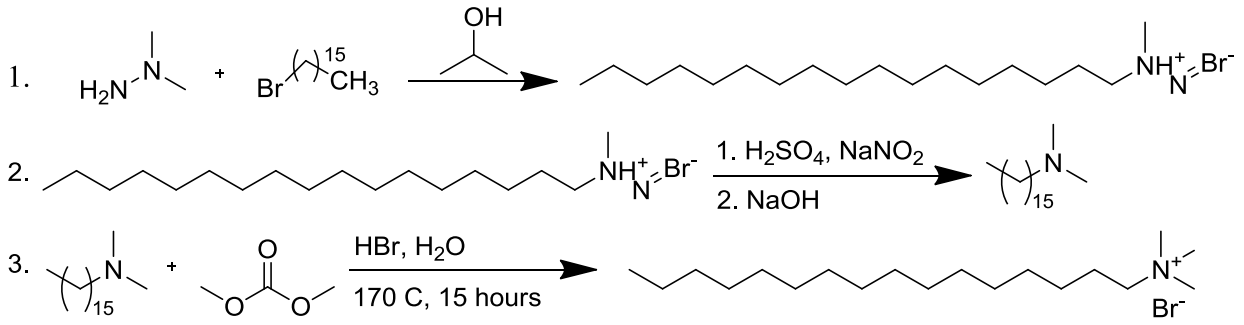
It is expected that, due to its much longer hydrophobic tail compared to SDS and SDM, HTAB will have a much lower hydrophilic-lipophilic balance value. It is also expected that HTAB will have a much lower CMC due to its increased surface area that the longer tails provide since this increases the ability of the surfactant to break the surface tension of the mother liquor. Furthermore, an HTAB molecule should have a much higher aggregation number than SDS and SDM due to its increased size, which greatly enhances the size of the surfactant micelles and thus the interior capacity for molecules. To verify the structure of the new surfactant and the results of the synthesis procedure, we report a number of characterizations performed using FT-IR, ^{13}C NMR, ^{1}H NMR, Raman spectroscopy, UV spectroscopy, and inductively coupled plasma mass spectrometry (ICP-MS). The use of cationic HTAB versus anionic SDS is expected to enhance the quality of the bands in SDS-PAGE when analyzing negatively-charged

glycoproteins. An SDS-PAGE procedure will be performed using both of the surfactants to analyze this. Finally, the potential use of HTAB as a coating for nanoparticles instead of the carcinogenic dimethylformamide will be explored. The nonpolar nature of the hydrocarbon tails in HTAB should provide at least some greater stability toward both strong acid and alkaline environments relative to dimethylformamide. This would greatly enhance the suitability for gold nanoparticle use in the human body and as a potential drug delivery carrier system.

Materials and Methods

Synthesis, Reagents, and Materials

The synthesis of HTAB is a multi-step process involving commercially-available 1,1-dimethylhydrazine and 1-bromohexadecane and takes approximately 30 hours, as illustrated in Scheme 2.



Scheme 2. Synthesis of Hexadecyltrimethylammonium Bromide (HTAB)

As previously mentioned, the synthesis does not use a catalyst. Other methods of preparation are possible. While this procedure is quite lengthy, it avoids complex procedures for removing the catalyst from products, which require special expertise and would still take additional time. The use of no catalyst has the added benefit of producing product in higher yield.

1,1-dimethylhydrazine (98% verified by assay), 1-bromohexadecane (97% by assay), *BioReagent* 2-propanol (99% by assay), ACS reagent sodium nitrite (97% by assay),

ReagentPlus dimethyl carbonate (99% by assay), and *BioXtra* sodium hydroxide (pellets, anhydrous, 98% by assay) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Optima-grade hydrobromic acid and optima-grade sulfuric acid were purchased from Fisher Chemical (Waltham, MA). Ultrapure water with a resistivity of 18.2 MΩ used in synthesis and preparation of materials was obtained from a Milli-Q Integral water filtration system (EMD Millipore Corporation, Billerica, MA).

Hydrophilic-Lipophilic Balance (HLB) Calculation

The hydrophilic-lipophilic balance (HLB) is a measure of the degree to which a surfactant molecule is hydrophilic or lipophilic. HLB was first described by Griffin[16,17] and later refined by Davies.[18] An HLB value for a given surfactant with molecular mass of the lipophilic portion, , and overall molecular mass, , is given by Equation 1 below.

—— **(1)**

While not a description of the efficiency of the surfactant, hydrophilic-lipophilic balance indicates how a particular surfactant or emulsifying agent will behave. It is an expression of the relative attraction of an emulsifier for water and oil, determined largely by the chemical composition and ionization characteristics of a given emulsifier. The use of hydrophilic-lipophilic balance as a nonionic surfactant parameter has been well established as a universal parameter.[19,20] Values less than 10 are generally water-insoluble while values greater than 10 are water-soluble. Wetting agents are typically in the 11-14 range, detergents in the 12-15 range, and solubilizing agents in the 16-20 range.[18] Hydrophilic-lipophilic balance was chosen as a behavior parameter over oil-water partition (KWO) and solubility parameters involving characterization of hydrogen bonding (symbolized by ΔO in most literature) since not all potential applications with hexadecyltrimethylammonium bromide are water-insoluble.

Critical Micelle Concentration (CMC) Calculation with UV-Vis Spectroscopy

The critical micelle concentration (CMC) of a surfactant is a range of concentrations above which virtually all additional surfactant molecules form micelles.[21] Micelle formation in an aqueous solution is a spontaneous process, which means that the Gibbs free energy of formation is negative. When added to a solution, a surfactant rapidly reaches an equilibrium between aggregated and free ambiphiles. The equilibrium constant is therefore given by Equation 2 where [M] is the molar concentration of aggregated ambiphiles and [S] the molar concentration of free ambiphiles. The number of moles of S in equilibrium with M is given by *n*.

(2)

Using thermodynamic principles and deriving the generic Gibbs free energy formula, the Gibbs free energy of S at standard temperature and pressure is given by Equation 3.[22]

(3)

This equation may be further simplified to Equation 4 where T is temperature and R the appropriate gas constant depending on the required units. Assuming no additional electrolytes are present in the system aside from the surfactant, and incorporating the Gibbs-Helmholtz equation, the critical micelle concentration is thus expressed using Equation 4, where the units are the same as in Equation 3, with an additional (2-p/n) term which describes counter-ions binding to the micelle.[22] This value is generally 0.2 for surfactants and is the value used in these calculations.[22]

(4)

Critical micelle concentration has been measured in previous works through UV-Vis spectroscopy, luminescence spectroscopy, and electrical conductivity.[23] In this work, UV-Vis

was used in experimental CMC determination. Measurements were made using a LAMBDA XLS+ UV/Vis Spectrophotometer (PerkinElmer Inc., Waltham, MA). Fluorescence spectroscopy, while the most precise of the three, was not chosen as HTAB lacks an aromatic complex that conveys vibrational bands, which are the basis for the method.[23] An enhancement of the absorption in a UV-Vis spectrum is observed at concentrations just above the critical micelle concentration. The UV-Vis method has been shown previously to be an accurate method for CMC determination in cationic, anionic, and nonionic surfactants.[23]

Aggregation Number Fluorescence Measurement and Calculation

Aggregation number was calculated using a method previously described by Tummino and Gafni.[24] This method is similar to those used in other works.[25] The value calculated in this work for critical micelle concentration and a measured density of 0.995 cm^3/g were used in aggregation number calculations. Steady-state fluorescence measurements were measured using a Fluorolog II (Spex Industries Inc., Edison, NJ). Fluorescence measurements are usually taken at ambient temperature, but since the Krafft point (temperature at which the solubility of a surfactant rises sharply and micelles begin to form in a solution) of HTAB is 22 °C, the measurement was taken instead at 30 °C.[26] The Förster distance, R_0, is the distance at which the energy transfer efficiency between a donor and acceptor is 50%.[24] This distance, in angstroms, is calculated according to Equation 5 below.

(5)

J is the spectral overlap integral, κ^2 the orientation factor between the donor and acceptor chromophores, Q_0 the fluorescence quantum yield of the donor in absence of the acceptor, and the refractive index, *n*.[24] The Förster distance provides a quantifiable way to describe molecular dynamics.[24]

Aggregation number was determined using this previously defined method involving fluorescence quenching. A partition coefficient is first calculated in this analysis according to Equation 6.

(6)

Having found this partition coefficient, the fluorescence measurements can be related to the partition coefficient using Equation 7. Values of α_m are found by subtracting the critical micelle concentration from total detergent monomer concentration and multiplying by the molecular weight and specific volume of the detergent.[24]

(7)

Using this same fluorescence ratio described above and assuming a partition of one quencher molecule completely quenches the fluorescence of the surfactant, we arrive at Equation 8. A plot of versus the micelle concentration has the slope -1/N where N is micelle concentration.[24] Using information for N and a known detergent concentration, the number of detergent monomers per micelle, the aggregation number, can be calculated.[24]

(8)

^{1}H and ^{13}C NMR Characterization

The proton NMR was collected at 90 MHz using a JEOL FX-900 (Jeol Corp., Tokyo, Japan), with the sample dissolved in cholorofom-d (Sigma-Aldrich, St. Louis, MO). The carbon NMR was collected using a Varian CFT-20 instrument (Varian Inc., Palo Alto, CA) with the sample again dissolved in chloroform-d (Sigma-Aldrich, St. Louis, MO). Tetramethylsilane (TMS) was used as the reference standard in both cases (Sigma-Aldrich, St. Louis, MO).

Additional characterization methods are provided in the appendix as captions below the respective spectra.

PAGE and Western Blotting Analysis of Glycoproteins with SDS and HTAB

SDS-PAGE analysis was performed with a Multiphor II (PerkinElmer Inc., Waltham, MA) using a previously defined procedure by Han and Chen with slight modification for the SDS-PAGE procedure.[27] Antibodies for blotting were purchased from Accurate (Westbury, NY) and culture gels were purchased from Life Technologies (Karlsruhe, Germany). These cultures were grown as Han and Chen suggested, with resistance to colchicine chosen as the staining target.[27] HTAB-PAGE analysis was performed similarly to the SDS-PAGE analysis with the cell cultures grown in identical fashion. HTAB-PAGE analysis used a procedure previously defined by Jovin[28] which allowed proper stacking of bands during staining. Gels in both analyses were prepared using a method by Rabilloud et al. with 37 μM methylene blue, 500 μM toluene disulfinate, and 25 μM diphenyliodonium chloride.[29] All required chemicals for these procedures were purchased from Fisher (Waltham, MA).

Manufacturing of Gold Nanoparticles Coated with HTAB

Reactant-free gold nanoparticles (5 nm in diameter) were purchased from Sigma-Aldrich (St. Louis, MO). To remove any potential existing coating, the nanoparticles were suspended in choloform and ethanol before centrifugation and sonication. This process was repeated two more times. Smolensky et al. first reported this stripping procedure for use with magnetite nanoparticles and we have adapted it here for gold nanoparticles.[30] Following this stripping procedure, the nanoparticles were submersed in a solution of dichloromethane and prepared HTAB before suspension in ultra-pure water and analysis using TMS microscopy. Results are shown in Figure 6. All chemicals in this process were purchased from Fisher (Waltham, MA).

Results and Discussion

^{1}H and ^{13}C NMR Spectroscopy Characterization Analysis

HTAB was first synthesized according to the synthesis procedure. Following synthesis, the compound was characterized using ^{1}H NMR and ^{13}C NMR spectroscopy; these results are shown in Figures 3 and 4 respectively.

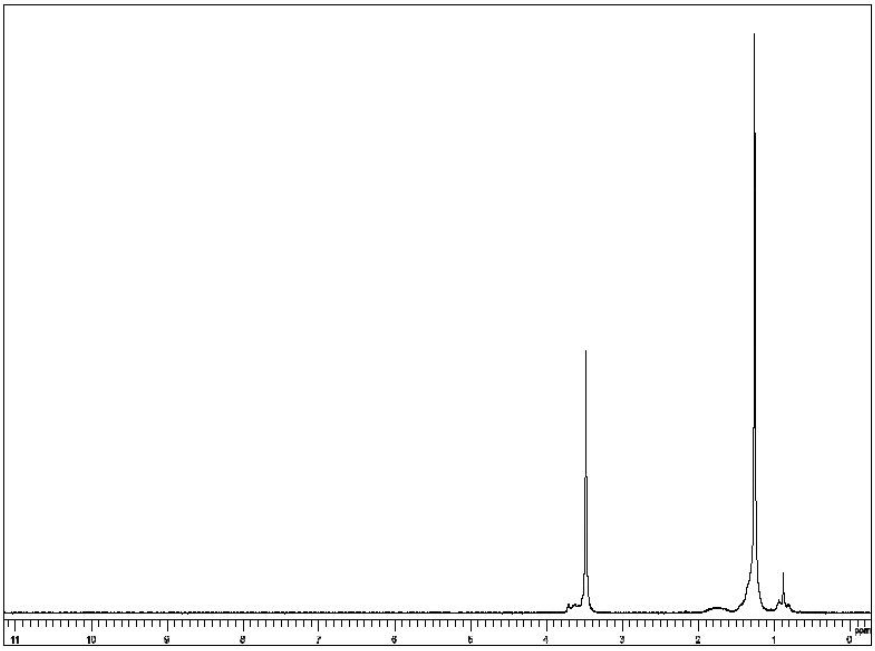


Figure 3. ^{1}H NMR of Hexadecyltrimethylammonium Bromide (HTAB)

The results of the ^{1}H NMR are as expected in the structure of the product, providing an indication that the final product is as desired. N-H absorptions are expected in the range of 1 – 5 ppm and alkyl absorptions of the hydrocarbon tail are expected around 1.3 ppm.[31] The amino protons should be downshifted a little bit, owing to electron withdrawing of nitrogen and thus further magnetic field exposure. Since proton NMR also provides information as to the relative number of protons in the molecule, it is expected that the area under the curve at 1.3 ppm should appear in an approximate 3:1 ratio to the area under the curve for N-H bonds absorption, since

there are 30 protons along the hydrocarbon chain and 11 attached on a carbon alpha to nitrogen. The spectra also matches this expectation, as there is a strong absorption around 1.3 ppm and a smaller absorption around 3.5 ppm.

The results of the ^{13}C NMR are also as expected in the structure of the product, providing another indication that the final product is as desired. The three methyl groups attached to the nitrogen atom are expected around 47.6 ppm.[31] One of the methyl groups is substituted with the alkyl chain, and the absorption for two carbons on the nitrogen should be downshifted a little bit. Alkyl absorptions occur in the 0 – 50 ppm range.[31] We see the typical splitting patterns on carbons around the nitrogen atom, as well as a multiplet typical of CH_2 groups that have neighboring groups on both sides in the hydrocarbon tail region. Bromine on an alpha atom to another carbon may also have some influence on some observed shifts, but the observation would be slight.

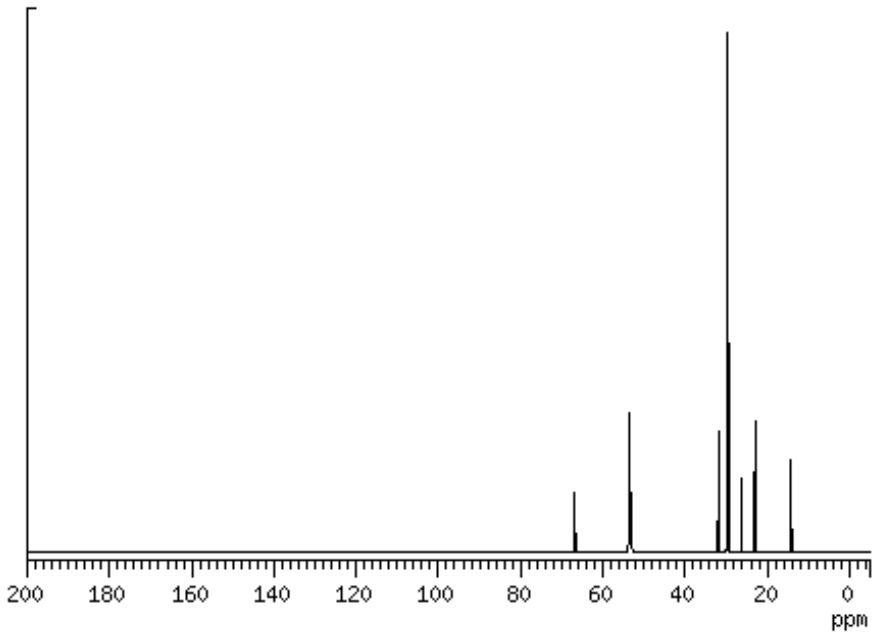


Figure 4. ^{13}C NMR of Hexadecyltrimethylammonium Bromide (HTAB)

Additional characterization spectra are available in the appendix, which provide further confirmation of the structure of the final product.

Analysis of Performance Characteristics

After calculations of the performance and behavior data, HTAB had a hydrophilic-lipophilic balance (HLB) of 10, which is immediately between what would be expected to be water-soluble and what would be expected to be water-insoluble. The value also makes the compound more of a wetting agent, compared to SDS and SDM, which fall under a solubilizing agent. The HLB values of these three compounds, as well as critical micelle concentration, aggregation number, and UV absorption values are provided in Table 1.

Table 1. Performance Characteristics of Hexadecyltrimethylammonium Bromide Compared with Other Cationic and Biologically-Active Detergents

Surfactant / Detergent	HLB	CMC	Aggregation Number	UV Abs.[a]	Price (USD 50g)
Hexadecyltrimethylammonium bromide (CAS No 57-09-0)	10	0.92 (1 mM)	170	0.06	$65.30
Sodium dodecyl sulfate (CAS No 151-21-3)[32,33]	40	7 – 10 mM	62	0.04	$64.30
Sodium deoxycholate monohydrate (CAS No 145224-92-6)[34,35]	16	2 – 6 mM	3 – 12	0.10	$1290.00

[a] UV absorption value reported at $\lambda = 260$ nm.

The critical micelle concentration of HTAB was calculated to be 0.92 (around 1 mM), which is much lower than the other two compounds used for comparison. This indicates a very low concentration of HTAB is needed for micelle formation, and the compound is thus a potent detergent. A calculated aggregation number shows HTAB to be very high compared to SDS and SDM. The measured UV absorption values used in calculating the aggregation number are also provided in Table 1. When comparing cost of manufacturing the three compounds, HTAB is estimated, based on the cost of starting materials and a slight markup for commercial supply, to

be very similar to SDS and much cheaper than SDM. The compound possesses a much lower CMC, which allows a significantly lower amount to be used to reach a desired effect. A very high aggregation number results in a high inner volume within the micelle, allowing the detergent to solubilize large molecules easily. These results also indicate that, compared to the other two detergents, HTAB strongly prefers large clusters in a micelle, as opposed to many, smaller clusters.

SDS-PAGE and HTAB-PAGE Comparison for Glycoprotein Analysis

As seen in Figure 5 below, blot lines following HTAB-PAGE analysis of glycoproteins are much more succinct and readable when compared to SDS-PAGE analysis. The repulsive attraction between the negatively-charged glycoproteins and the anionic SDS has thus improved with the introduction of cationic HTAB. Given the much higher aggregation number of HTAB versus SDS in combination with these PAGE results, HTAB also tends to have the advantage in blotting when dealing with larger proteins and protein complexes, as there are many more molecules of surfactant per micelle in HTAB than there are in SDS. SDM is nonionic and may also show distinct lines, but is more disfavorable with large proteins (lower aggregation number).

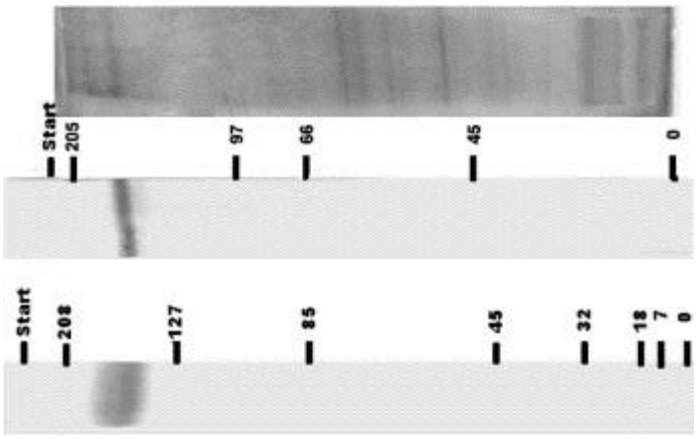


Figure 5. Blots of Plasma Membrane Samples Following SDS-PAGE (Bottom) and HTAB-PAGE (Middle); Top Depicts Silver-Stained HTAB-PAGE Gel

TMS Microscopy Analysis of HTAB-Prepared Gold Nanoparticles

In the final stage of analysis of HTAB as a potential surfactant, the effect of coating gold nanoparticles with HTAB was explored. TMS microscope images of various morphologies of gold nanoparticles prepared using HTAB coating are shown in Figure 6. The nanorod morphology is the most commonly used for gold nanoparticles and is shown in Figure 6D. When compared to the gold nanoparticles prepared with dimethylformamide, it appears HTAB adequately prevents the nanoparticles from aggregating. The results show HTAB to do this in similar fashion to dimethylformamide and establish HTAB as a potential coating for nanorods.

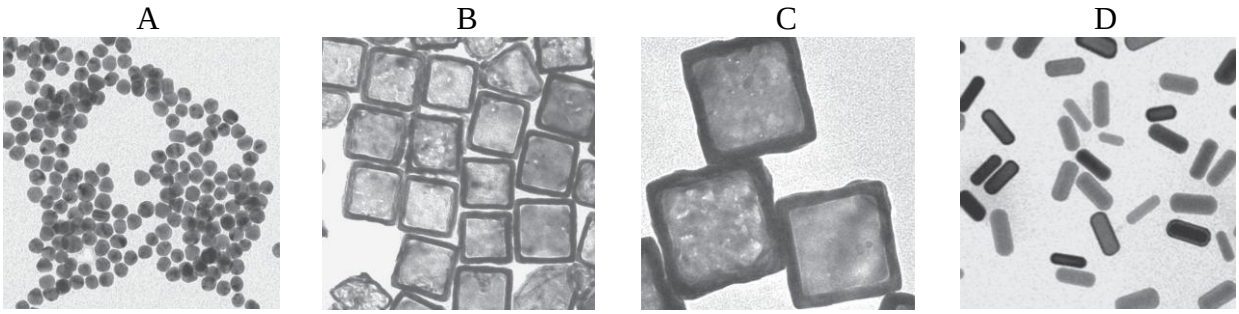


Figure 6. TMS Microscope Images of Gold Nanoparticles Following a Manufacturing Process with Hexadecyltrimethylammonium Bromide (HTAB); A-C Show Various Morphologies in Different Aspect Ratios while D Shows the Common Nanorod Morphology

With the cationic HTAB, the negative charge experienced with the gold nanoparticles upon binding should be more stabilized and should allow more control over the nanoparticles versus coating with an anionic or nonionic surfactant. Further *in vitro* analysis is needed to examine the behavior of HTAB-Au nanoparticles before implementation into a drug delivery system.

Conclusion

From our characterization data, it is evident that the synthesis procedure was successful and produced the desired product. This procedure did not involve the use of catalyst, allowing higher product yield and the avoidance of complex catalyst-product separation methods which

require additional expertise and time. When compared to the similar surfactants, sodium dodecyl sulfate and sodium deoxycholate monohydrate, hexadecyltrimethylammonium bromide had a much higher aggregation number, allowing more association ability with larger molecules. With a lower critical micelle concentration, smaller amounts of HTAB are necessary to form micelles. This feature, along with the relatively inexpensive synthesis procedure, shows HTAB to be a prime candidate for industrial use. When compared with SDS-PAGE, HTAB-PAGE also appeared to dissipate the like charge interferences seen during glycoprotein analysis. More distinct and readable lines were produced following HTAB-PAGE than with analysis using SDS-PAGE. The advantage factor for HTAB-PAGE analysis also increases in PAGE-based analyses due to the higher aggregation number of HTAB. The increased length of the hydrocarbon tail (which is also responsible for the lower HLB value) in HTAB allows greater van der Waals interactions with larger proteins and thus a greater "association ability" with large glycoproteins.

HTAB was also demonstrated to be an adequate anti-aggregation coating for use in gold nanoparticle manufacturing. The positively-charged HTAB molecule allows greater stabilization of the negative charge the nanoparticles possess upon binding to a target surface and provides a reasonable, safe alternative to carcinogenous dimethylformamide. All of our results support our original hypothesis and imply that hexadecyltrimethylammonium bromide is a widely-applicable, inexpensive, safe, and high-potential cationic surfactant.

Supplemental Material Available: The appendix contains a detailed description of the synthesis of HTAB and spectroscopic characterization by Fourier-Transform IR spectroscopy (FT-IR), UV-Vis spectroscopy, proton NMR, carbon NMR, Raman spectroscopy, and mass spectrometry. The appendix can be obtained by contacting the authors.

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Supporting Information

Hexadecyltrimethylammonium Bromide: A Novel Cationic Detergent

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Synthesis of Hexadecyltrimethylammonium Bromide

Hexadecyltrimethylammonium bromide is synthesized according to a multi-step synthesis procedure from commercially-available 1,1-dimethylhydrazine and 1-bromohexadecane which are first combined in 2-propanol. This first step results in 91% yield of 1-hexadecyl-1,1-dimethylhydrazonium bromide. In the second step, aqueous sulfuric acid and $NaNO_2$ are added to the 1-hexadecyl-1,1-dimethylhydrazonium bromide followed by the addition of sodium hydroxide in water after the sulfuric acid and $NaNO_2$ have had time to react. This step results in 73% yield of N,N-dimethylhexadecylamine. The approximate synthesis time for this first series of steps is around 15 hours.

The following series of steps are the key development. The produced N,N-dimethylhexadecylamine is added to dimethyl carbonate. Concentrated hydrobromic acid is combined into the reaction flask using H_2O solvent at room-temperature and the reaction is heated at 170 °C for 15 hours, resulting in hexadecyltrimethylammonium bromide after a total synthesis time of 30 hours. While somewhat lengthy, the synthesis manages to avoid the use of a catalyst in the final stages, which is particularly useful when the separation of catalyst from products is difficult and when the catalyst cannot be reused. The overall result is a higher product yield. Reagents and solvents used in the synthesis are also relatively inexpensive.

FT-IR Spectrum of Hexadecyltrimethylammonium Bromide

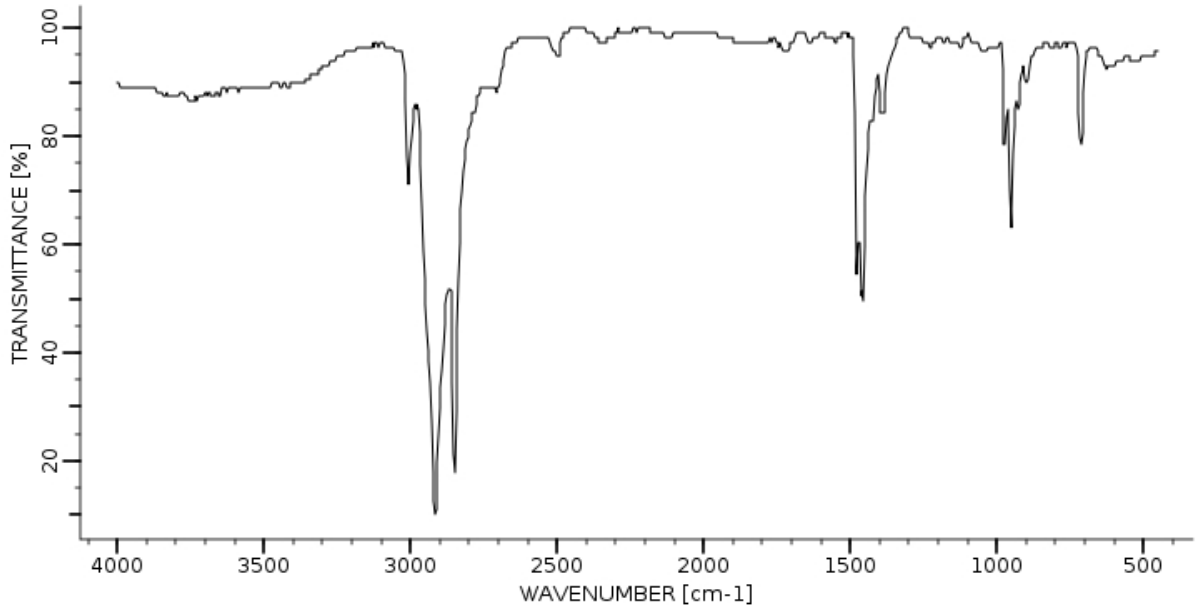

Figure S1. FT-IR spectrum of hexadecyltrimethylammonium bromide taken in a KBr pellet using a Nicolet Magna-IR 750 Spectrometer Series II

UV-Vis Spectrum of Hexadecyltrimethylammonium Bromide

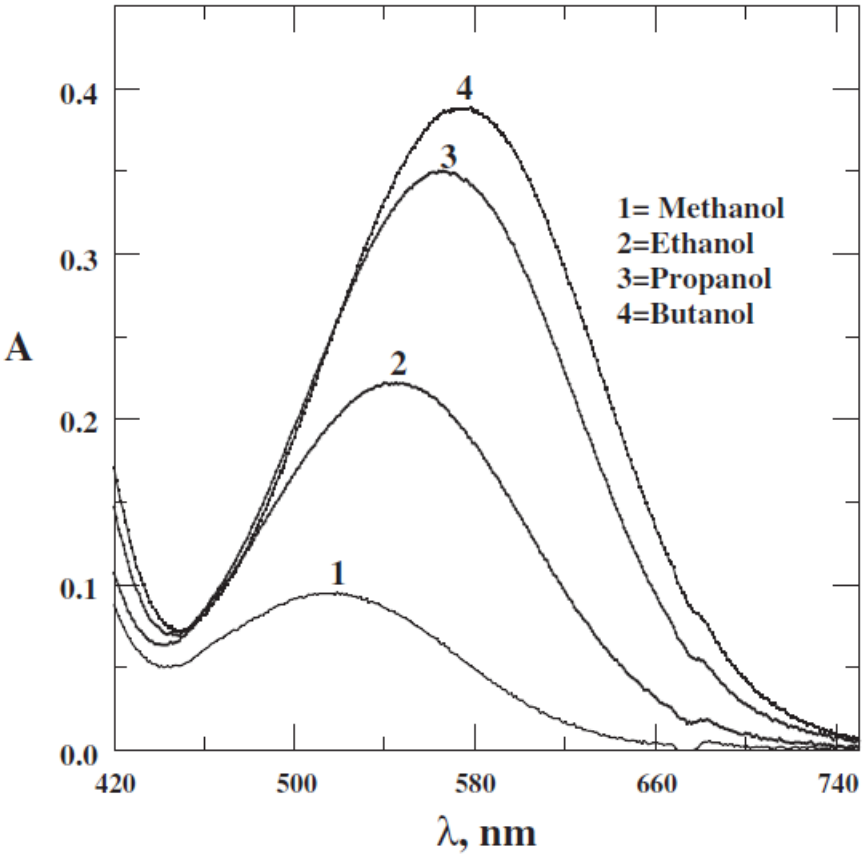

Figure S2. UV-Vis spectrum of hexadecyltrimethylammonium bromide with different solvents and their effects; spectrum collected using PerkinElmer LAMBDA XLS+ UV/Vis Spectrophotometer

Proton (^{1}H) NMR Spectrum of Hexadecyltrimethylammonium Bromide

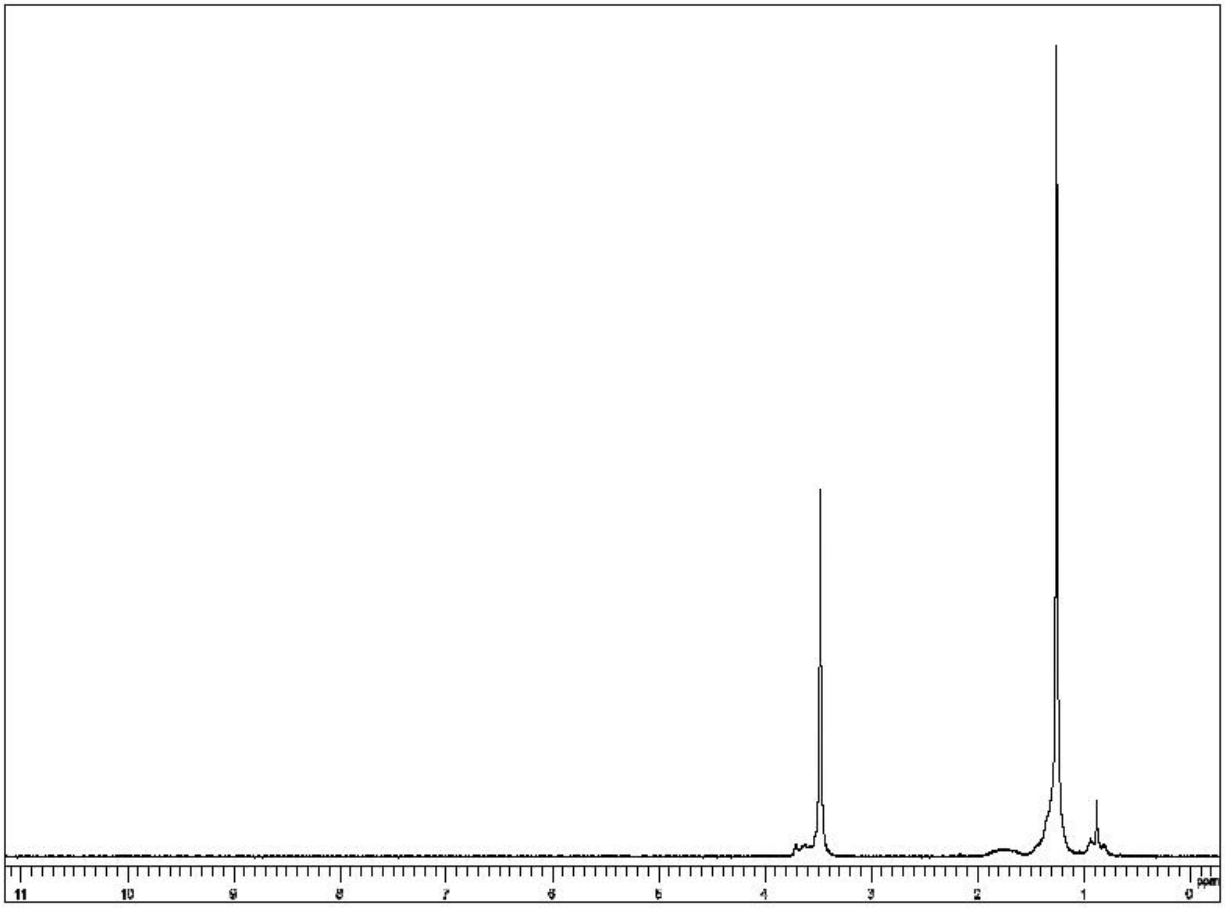

Figure S3. ^{1}H NMR spectrum of hexadecyltrimethylammonium bromide obtained at 90 MHz using a JEOL FX-90Q; sample was dissolved in chloroform-d and referenced against tetramethylsilane (TMS) standard

Carbon (^{13}C) NMR Spectrum of Hexadecyltrimethylammonium Bromide

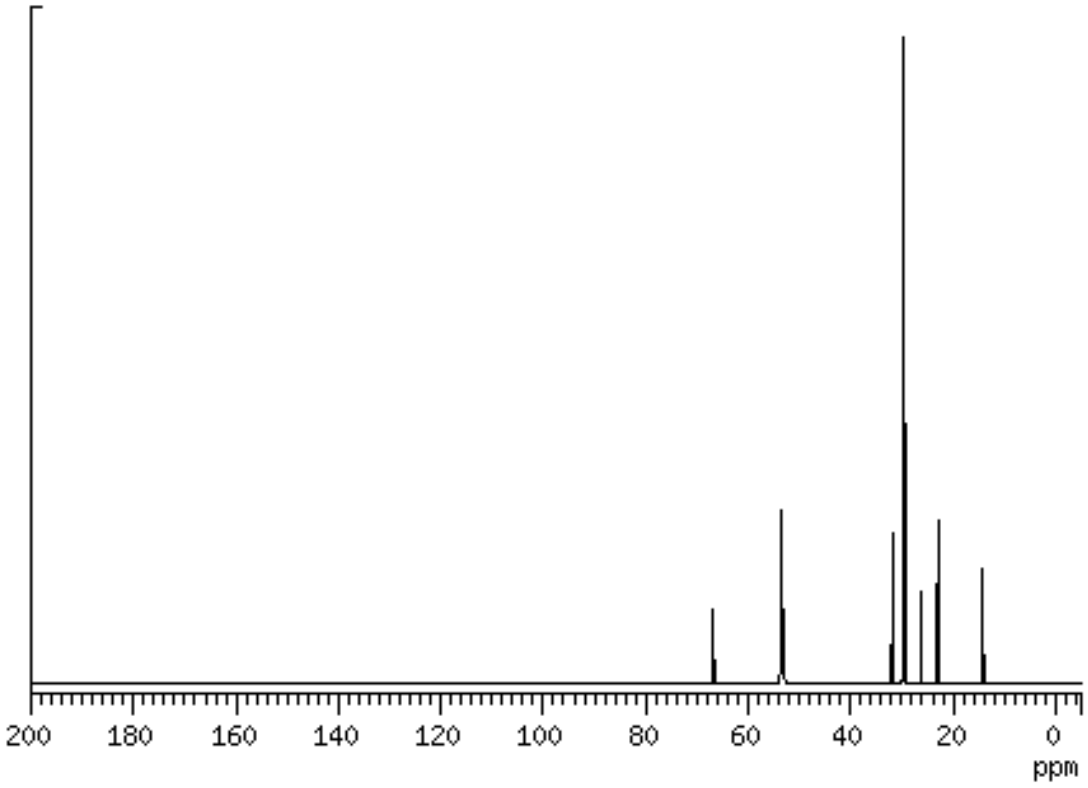

Figure S4. ^{13}C NMR spectrum of hexadecyltrimethylammonium bromide obtained using a Varian CFT-20 instrument; sample was dissolved in chloroform-d and referenced against tetramethylsilane (TMS) standard

Raman Spectrum of Hexadecyltrimethylammonium Bromide

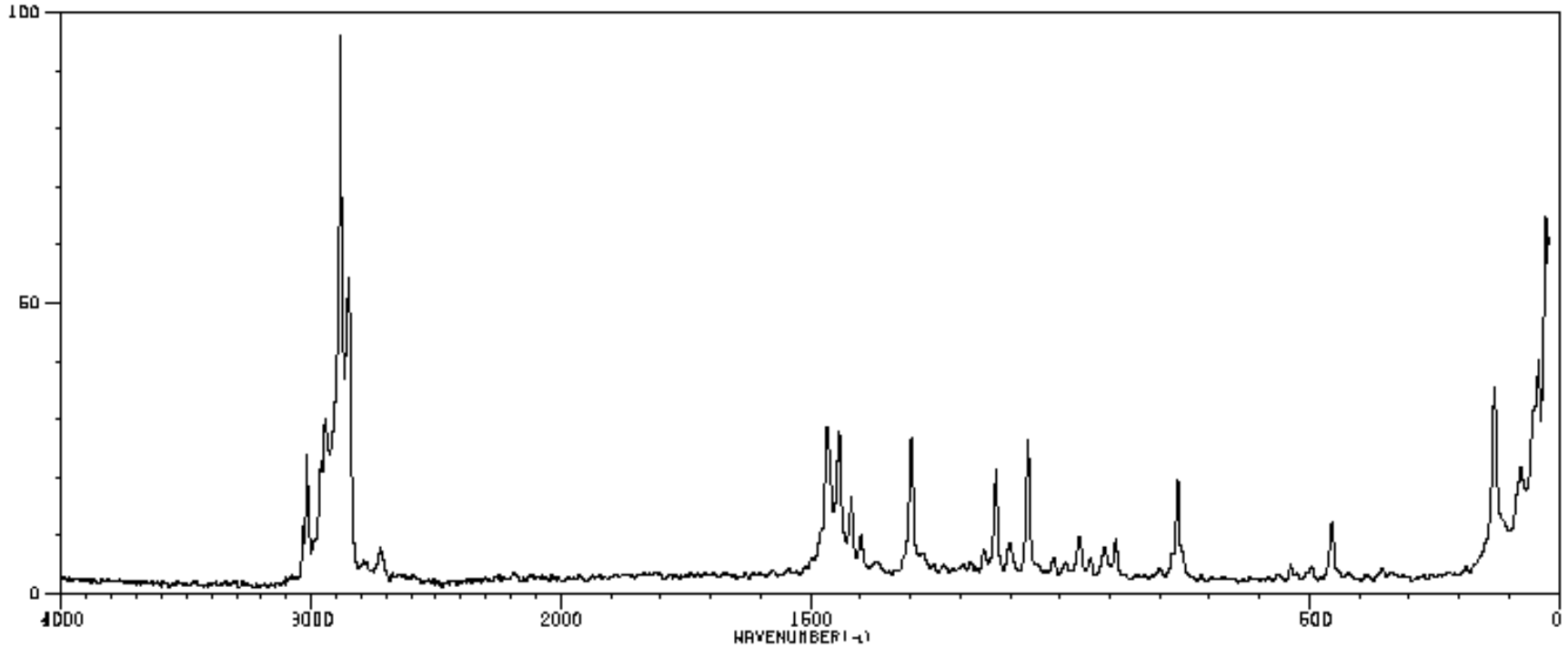

Figure S5. Raman spectrum of hexadecyltrimethylammonium bromide collected using Bruker Optics MultiRAM Stand Alone FT-Raman Spectrometer

Mass Spectrum of Hexadecyltrimethylammonium Bromide

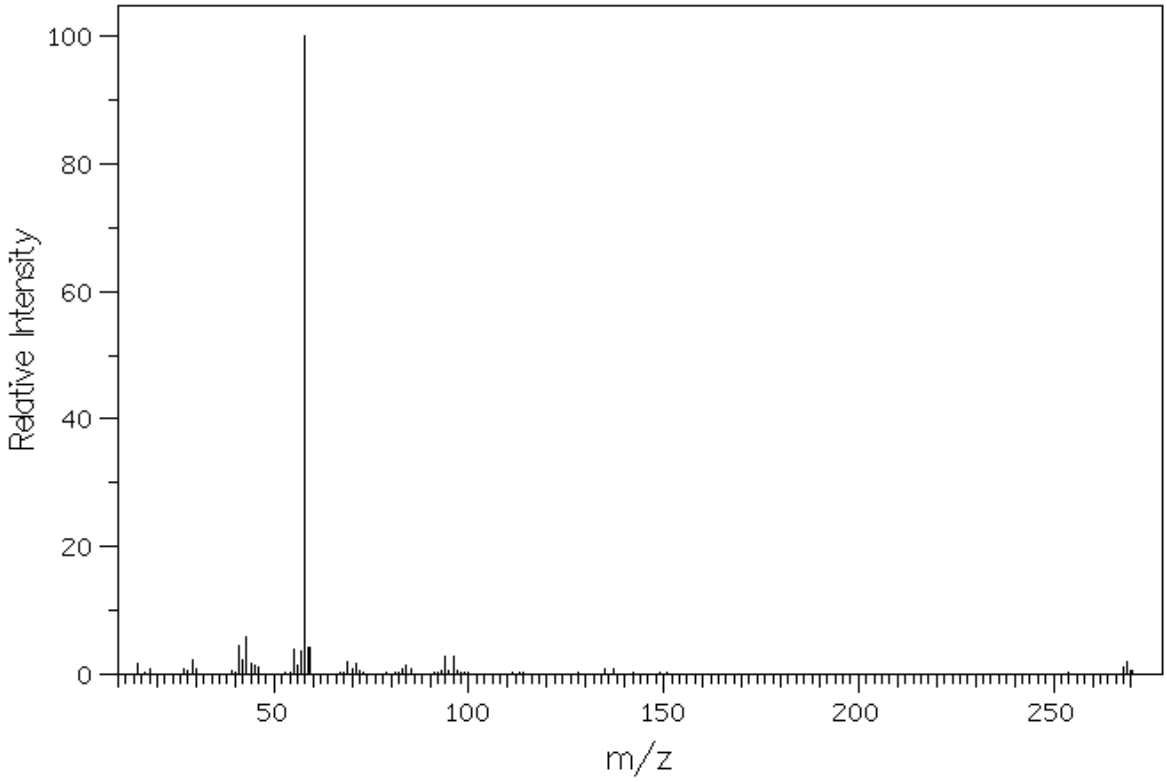

Figure S6. Mass spectrum of hexadecyltrimethylammonium bromide obtained using a PerkinElmer 300X NexION Quadrupole Inductively Coupled Plasma Mass Spectrometer (Q-ICP-MS) with a cyclonic spray chamber sample introduction system; samples were stabilized in concentrated in nitric acid and diluted to 10 mL with 18.2 MΩ H_2O

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